Forensic Science International: Genetics 5 (2011) e71–e77

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Forensic Population Genetics – Short Communication

Diversity of 15 human X chromosome microsatellite loci in Polish population

Sylwia Łuczak [a], Urszula Rogalla [a], Boris A. Malyarchuk [b], Tomasz Grzybowski [a,*]

[a] The Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum, Institute of Forensic Medicine, Department of Molecular and Forensic Genetics, Bydgoszcz 85-094, Poland
[b] Institute of Biological Problems of the North, Far-East Branch of the Russian Academy of Sciences, Magadan 685000, Russia

ARTICLE INFO

Article history:
Received 16 May 2010
Received in revised form 20 December 2010
Accepted 24 December 2010

Keywords:
X chromosome
STR
Phylogeny
Balancing selection
Polish population

ABSTRACT

X-STR analysis is a powerful tool in both phylogeny reconstruction and forensic investigation. Hereby, we provide new population data concerning 15 X-STR loci (included in commercially available typing kit Mentype Argus X-8 (Biotype AG, Dresden, Germany) (DXS10135, DXS8378, DXS7132, DXS10074, HPRTB, DXS10101, DXS10134 and DXS7423) and another seven (DXS6807, DXS9898, DXS101, DXS7424, DXS7133, DXS8377 and DXS10011) that were previously described by Poetsch et al. [1] obtained from a sample of 311 individuals from Poland and compared to the results previously obtained from other populations of European, Asian and African origin [2–4]. Numerous experiments seem to prove that X-STRs are valuable markers for human identification, kinship testing and even phylogenetic research – thus serving as a complement for autosomal microsatellites, Y-STRs and mtDNA [5–7].

1. Population

311 samples (159 female and 152 male) from unrelated individuals inhabiting Kuyavia-Pomerania region in central-northern Poland were included in this study. As Polish population, in general, seems to be quite homogenous, as proved by other markers' analyses [8–10], Kuyavia-Pomeranians may be treated as representative sample of Poles. The study was approved by Nicolaus Copernicus University's Bioethical Commission in Bydgoszcz (Approval No. KB/201/2006).

2. DNA extraction

Genomic DNA extraction from blood or buccal swabs was performed by the phenol–chloroform method.

2.1. PCR

The multiplex test system Mentype Argus X-8 kit was used to amplify 8 loci in accordance to the manufacturer's instructions. Seven remaining loci were amplified with primers described previously [1]. PCRs were performed separately for each locus in GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). Reaction mix (final volume of 12.5 μl) contained 0.5 μl DNA, 0.5× PCR buffer, 2 mM $MgCl_2$, 0.1 mM dNTP each and 1 U of GoTaq Flexi polymerase (Promega, Madison, WI, USA). Thermal cycling conditions were not equal for all loci. Those were separated into two groups. DXS9898, DXS6807, DXS101 were enclosed in the first group that required 10 s of initial denaturation in 95 °C and subsequent 30 min of denaturation in 94 °C, 105 min of annealing in 61 °C and 60 min of elongation in 72 °C repeated in 30 cycles followed by final elongation in 72 °C for 10 min. Second group, consisting of DXS7133, DXS10011, DXS7424 and DXS8377 was typed under the cycling conditions given above, except for the annealing temperature and time, which in this case were 62 °C for 90 min.

3. STRs typing

The PCR products of Mentype Argus X-8 were separated and detected with ABI3100 instrument (Applied Biosystems, Foster City, CA, USA) and the alleles were typed automatically using the Genotyper v.3.7 and GeneMapper ID v.3.2.1 software (Applied Biosystems) with reference to the allelic ladders included in the multiplex kit. Remaining alleles were identified on the basis of female control DNA 9947A size (Promega) that were ascribed with alleles' names. Alleles' nomenclature was reconstructed on the basis of the literature data that concerned structure of specific sequence variants [1,11,12].

4. Quality control

The Department of Molecular and Forensic Genetics has participated in all GEDNAP proficiency tests for 9 years, always

* Corresponding author at: The Nicolaus Copernicus University, Ludwik Rydygier Collegium Medicum, Institute of Forensic Medicine, Department of Molecular and Forensic Genetics, Skłodowskiej-Curie 9, Bydgoszcz 85-094, Poland.
Tel.: +48 52 585 35 49; fax: +48 52 585 35 53.
E-mail addresses: sylwia-luczak@o2.pl (S. Łuczak), urszula.rogalla@cm.umk.pl (U. Rogalla), malyarchuk@ibpn.ru (B.A. Malyarchuk), tgrzyb@cm.umk.pl (T. Grzybowski).

1872-4973/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.fsigen.2010.12.009

achieving positive results. Moreover the Department was granted current quality certificate issued by the Polish Society of Forensic Medicine and Criminology. All the lab work is performed according to the internal control procedures. The presented study required usage of specified kit DNA control withal.

5. Data analysis

Locus-specific allele frequencies were estimated using Microsatellite Tools for Excel [13] while genotype and haplotype frequencies were calculated directly. Analysis of the conformity with Hardy–Weinberg equilibrium (HWE) was assessed by the means of Arlequin v.3.1 package [14] and corrected using Hochberg's method [15]. The same software was applied to selective neutrality testing using the Ewens–Watterson algorithm as well as linkage disequilibrium (LD) and Fst analysis. In addition, polymorphic information content (PIC), power of discrimination and power of exclusion for female samples were calculated with the use of PowerStatsV12 v.2 software (Promega, USA). Expected heterozygosity (HET) and mean exclusion chance (MEC) were also assessed [16–18]. PD for men was calculated on the basis of the formula proposed by Desmarais et al. [18] using Excel spreadsheet (Microsoft). On the basis of haplotypes from males in Polish population (present study), Japanese [2], Swedish [3], Hungarian [4] and Ghanaian [2], haplotype networks were constructed for linked loci included in the Mentype Argus X-8 kit, aiming depiction of their reciprocal phylogenetic relationships. The analysis was carried out with the use of Network v.4.5.1.0 software (Fluxus Technology Ltd.). In reconstruction the MJ algorithm has been used [19].

6. Results

Allele frequencies and statistical parameters' values for 15 X-STR loci in Polish population are shown in Tables 1 and 2. Table 3 summarizes expected and observed homozygosity for 15 X-STR loci in Polish population based on Ewens–Watterson test. Haplotypes' network for DXS10074–DXS7132 pair of loci is depicted in Fig. 1.

Fst analysis (Table 4.) revealed significant differences between populations under study (Polish, Japanese, Ghanaian, Hungarian and Swedish), which could result not only from geographic distances between them but also from specific demographic history. Gametic association in Polish population was measured by means of pairwise linkage disequilibrium testing between all pairs of the Argus X-8 loci in male samples (Table 5.).

Our data does not provide any evidence of linkage disequilibrium between loci within linkage groups analyzed, besides DXS8378 and DXS10135 pair. This result is in concordance with the data obtained previously for several populations of both European and Asian origin [3,20,21]. Simultaneously, it is in opposition with the LD results based on logarithm of the odds (LOD) scores in large number of three-generation German families [2]. While one cannot exclude recombination between loci included in the Argus X-8 kit [20], it is also possible that high mutation rate in X-STRs broke linkage disequilibrium between some loci.

7. Other remarks

7.1. Forensic utility

Comparison of the allelic range observed in Polish population with the data obtained for other populations led to conclusion that alleles revealed in Polish sample are highly concordant with those present in other population samples. The only exception was allele 4 in DXS10074 observed so far in Polish population only. It is worth noting, however, that the manufacturer of the Mentype Argus X-8 kit points to the possibility of occurrence of this allele. In most cases, the most frequent alleles of Poles are simultaneously the most frequent ones in general population. On the contrary – rarest alleles are common only in 3 loci for all populations. The most polymorphic locus of all is DXS10011, followed by DXS10135, DXS8377 and DXS10101. The most heterozygous marker is DXS10135, followed by DXS10011 and DXS8377.

Overall, our results show that all of the described loci may be of great use in forensic genetics. The only reservation is associated with DXS6807 and DXS10011, which do not conform the HWE. Deviations of the allele distribution from the HWE were also observed occasionally in other population studies including different X-STR markers [22], being explained by the possible presence of null alleles or peculiarities of some population groups analyzed [23,24]. As for DXS6807 and DXS10011 analyzed in our study, typing results were verified very carefully, and no excess of homozygotes, usually resulting from the null alleles, was observed. It is worth noting, however, that both DXS6807 and DXS10011 contain complex repeats and DXS10011 in particular is a very complex marker exhibiting many interalleles and structural variants within alleles of identical length [25,26]. In accordance with other studies, only one DXS10011 allele in our population sample exceeded a frequency value of 0.09 (Table 1). Due to the very high number of alleles at this locus, comparison of the estimated and the expected heterozygosity may occasionally result in deviation of the allele distribution from the HWE [25]. Alternatively, but less plausibly, hidden population substructure could lead to violation of the HWE at some loci, including DXS6807 and DXS10011.

All loci have high or very high polymorphic value and thus can be used in broad range – from personal identification, paternity testing, forensic cases to phylogenetic research.

7.2. Phylogeny analyses

Statistical calculations made for each X-STR locus have shown conformity of allele frequency distribution in Polish population with Hardy–Weinberg equilibrium for most of loci. In order to define mechanisms responsible for shaping X-STR loci diversity, both descriptive and phylogenetic statistical analyses were performed. Possibility of acting of selective pressure was verified by the Ewens–Watterson homozygosity test (Table 3). Critical *p*-values lower than 0.025 obtained for 6 loci (DXS9898 = 0.019, DXS101 = 0.007, DXS10011 = 0, DXS8377 = 0, DXS10101 = 0, DXS10135 = 0) suggest that frequency-dependent balancing selection might be involved. Moreover, in locus DXS7423 critical *p*-value exceeds boundary 0.025 only slightly, reaching 0.028. Together with low significance level values observed for the remaining loci (between 0.072 and 0.36), this points at possibility of balancing selection being the main force involved in increasing Polish population's diversity. Nevertheless it does not seem probable that STR loci located beyond coding region are under direct selective pressure. These markers are assumed to be neutral and their diversity may increase as a result of selective pressure acting on different loci tightly linked to these STRs. Selection increases diversity of chromosome X fragments that are under its direct pressure and simultaneously leads to accumulation of diversity in loci which are localized nearby (rarely separated from regions under selection due to low rate of recombination). Hence, evolutionary pedigree of these neutral regions is much longer than in more distal fragments, which results in much higher diversity of the former. Similar effect of frequency-dependent balancing selection was observed in other regions of the human genome: beta-defensin genes (*DEFB*) [27], intron 5 region of the *LMBR1* gene [28], *HLA* [29], *ABO* [30], ornithine decarboxylase

Table 1
Allele frequencies and some statistical parameters values for 15 X-STR loci in Polish population. Indexes f and m stand for female and male, respectively. HV represents HWE *p*-value corrected with Hochberg's method. MEC_{Kr} – according to [15], MEC_{Ki} – [16], and MEC_{Des} – [17].

	Locus								
Allele	DXS7133	DXS7424	DXS9898	DXS6807	DXS8378	HPRTB	DXS7423	DXS7132	DXS10074
4									0.0021
6	0.0043								
7	0.0128								0.0638
8	0.0319				0.0043				0.1319
9	0.4468		0.283		0.0149	0.017			0.0085
10	0.1404	0.0043	0.0106		0.366	0.0021			
11	0.3149	0.0021	0.2043	0.4362	0.3042	0.0957		0.0085	0.0021
11.2						0.0043			
12	0.0362	0.0362	0.2617	0.0298	0.266	0.3872		0.0851	
13	0.0106	0.0787	0.1787	0.0213	0.034	0.3192	0.1	0.2894	0.0043
14	0.0021	0.2191	0.0553	0.2765	0.0085	0.1362	0.3128	0.3681	0.0106
15		0.2745	0.0064	0.1894	0.0021	0.0298	0.4064	0.2149	0.0681
16		0.2553		0.0213		0.0085	0.1574	0.0319	0.217
17		0.0979		0.0255			0.0234	0.0021	0.2448
18		0.0191							0.1575
19		0.0128							0.0787
20									0.0106
PIC_f	0.61	0.75	0.74	0.64	0.73	0.68	0.65	0.67	0.82
PD_f	0.834	0.916	0.899	0.853	0.896	0.879	0.862	0.881	0.82
PD_m	0.708	0.815	0.776	0.694	0.693	0.714	0.681	0.741	0.823
HET_{exp}	0.665	0.7843	0.7755	0.696	0.7056	0.7243	0.7084	0.7188	0.8436
HET_{obs}	0.6289	0.7799	0.7925	0.5535	0.7044	0.7044	0.6792	0.6352	0.8679
HWE p-value	0.3724	0.7403	0.0627	0	0.5047	0.6454	0.259	0.4455	0.0971
HV	0.7403	0.7403	0.627	0	0.7403	0.7403	0.7403	0.7403	0.7403
MEC_{Kr}	0.4178	0.5551	0.5429	0.4142	0.4403	0.4647	0.4307	0.4818	0.6721
MEC_{Ki}	0.6159	0.7325	0.7258	0.6204	0.6453	0.6556	0.632	0.6763	0.812
MEC_{Des}	0.6159	0.7336	0.7258	0.6083	0.6453	0.6544	0.632	0.6763	0.812

	Locus		
Allele	DXS10011	DXS10101	DXS10134
25.2		0.0021	
26		0.0043	
26.2	0.0043	0.0106	
27		0.017	
27.2	0.0043	0.0298	
28	0.0021	0.0404	
28.2	0.017	0.0851	
29		0.0851	
29.2	0.0191	0.0894	
30		0.0915	0.0043
30.2	0.0085	0.117	
31	0.0255	0.1129	0.0021
31.2	0.017	0.0936	
31.3	0.0021		
32	0.0533	0.0809	0.0106
32.1	0.0043		
32.2	0.0255	0.0553	
33	0.0958	0.0383	0.0532
33.1	0.0021		
33.2	0.0191	0.0255	
34	0.0255	0.0106	0.1085
34.1	0.0191		
34.2	0.0277	0.0085	
35	0.034	0.0021	0.1873
35.2	0.0533		
35.3			0.0277
36	0.0128		0.2085
36.1	0.0149		
36.2	0.0383		0.0149
37	0.017		0.1426
37.2	0.0617		0.0106
37.3			0.0085
38	0.034		0.0681
38.2	0.0362		
38.3			0.0149
39	0.0213		0.0213
39.2	0.0489		0.0021
39.3			0.0383
40	0.0319		0.0106
40.2	0.0404		
40.3			0.0234
41	0.0362		0.0021

	Locus	
Allele	DXS101	DXS10135
15	0.017	0.0021
16	0.0021	0.0021
17	0.0085	0.0106
17.1		0.0021
18	0.0915	0.0191
18.1		0.0064
19	0.0638	0.0532
19.1		0.0234
20	0.0106	0.0574
20.1		0.0234
21	0.0255	0.0937
21.1		0.0191
22	0.0234	0.0596
22.1		0.0277
22.2		0.0021
23	0.0681	0.0745
23.1		0.0234
24	0.217	0.0979
25	0.1469	0.0788
25.1		0.0106
26	0.1575	0.0723
26.1		0.0043
27	0.0894	0.0702
28	0.0447	0.0638
29	0.0255	0.0213
30	0.0085	0.034
31		0.0213
32		0.0128
33		0.0085
34		0.0043

	Locus
Allele	DXS8377
39	0.0021
40	0.0106
41	0.0149
42	0.0213
43	0.0489
44	0.0319
45	0.0404
46	0.0617
47	0.1192
48	0.1149
49	0.1
50	0.1128
51	0.0979
52	0.0745
53	0.066
54	0.0447
55	0.0191
56	0.0064
57	0.0106
58	0.0021

Table 1 (*Continued*)

	Locus				Locus			Locus
Allele	DXS10011	DXS10101	DXS10134	Allele	DXS101	DXS10135	Allele	DXS8377
41.3			0.0255					
42	0.0404							
42.2	0.0213							
42.3			0.0128					
43	0.034							
43.3			0.0021					
44	0.0255							
45	0.0064							
46	0.0085							
47	0.0043							
48	0.0043							
49	0.0021							
PIC_f	0.96	0.91	0.87		0.88	0.94		0.91
PD_f	0.991	0.91	0.968		0.97	0.94		0.982
PD_m	0.96	0.917	0.88		0.85	0.934		0.92
HET_{exp}	0.9597	0.9194	0.8768		0.8885	0.9444		0.9177
HET_{obs}	0.8654	0.805	0.805		0.9057	0.9371		0.8679
HWE *p*-value	0	0.0004	0.0054		0.2774	0.0546		0.0847
HV	0	0.0052	0.0648		0.7403	0.6006		0.7403
MEC_{Kr}	0.9127	0.815	0.7467		0.7648	0.8425		0.831
MEC_{Ki}	0.9544	0.901	0.8591		0.8709	0.9164		0.9097
MEC_{Des}	0.9555	0.9021	0.8602		0.8709	0.9174		0.9118

Table 2
Haplotype frequencies in four linkage groups calculated for 152 male chromosomes.

DXS8378	DXS10135	Haplotype frequencies	DXS7132	DXS10074	Haplotype frequencies	HPRTB	DXS10101	Haplotype frequencies	DXS7423	DXS10134	Haplotype frequencies
Allele	Allele		Allele	Allele		Allele	Allele		Allele	Allele	
9	21	0.0066	11	16	0.0066	9	30.2	0.0066	13	33	0.0066
9	22	0.0066	12	8	0.0263	9	31	0.0066	13	35	0.0066
9	28	0.0066	12	9	0.0066	11	27.2	0.0066	13	35.3	0.0066
10	18	0.0132	12	15	0.0066	11	28	0.0132	13	36	0.0263
10	19.1	0.0263	12	16	0.0197	11	28.2	0.0132	13	36.2	0.0132
10	20	0.0328	12	17	0.0329	11	30.2	0.0132	13	37	0.0066
10	20.1	0.0197	12	18	0.0132	11	31	0.0197	13	38	0.0066
10	21	0.046	12	20	0.0066	11	31.2	0.0066	13	39	0.0132
10	21.1	0.0132	13	7	0.0197	11	32.2	0.0132	14	33	0.0066
10	22	0.0263	13	8	0.0066	11	34	0.0066	14	34	0.0394
10	22.1	0.0197	13	15	0.046	12	27	0.0197	14	35	0.0525
10	22.2	0.0066	13	16	0.0592	12	27.2	0.0132	14	36	0.0394
10	23	0.0394	13	17	0.0854	12	28	0.0263	14	36.2	0.0066
10	24	0.0657	13	18	0.0592	12	28.2	0.0263	14	37	0.046
10	25	0.0328	13	19	0.0066	12	29	0.0394	14	37.2	0.0066
10	26	0.0132	14	7	0.0263	12	29.2	0.0591	14	37.3	0.0066
10	26.1	0.0066	14	8	0.046	12	30	0.0657	14	38	0.0132
10	27	0.0132	14	11	0.0066	12	30.2	0.0329	14	38.3	0.0066
10	28	0.0066	14	15	0.0066	12	31	0.046	14	39	0.0066
10	30	0.0066	14	16	0.0592	12	31.2	0.0066	14	39.2	0.0066
10	34	0.0066	14	17	0.1118	12	32	0.0263	14	39.3	0.0066
11	19	0.0066	14	18	0.0329	12	32.2	0.0263	14	41	0.0066
11	20	0.0132	14	19	0.0395	12	33	0.0132	15	30	0.0066
11	20.1	0.0066	15	7	0.0066	13	25.2	0.0066	15	33	0.0197
11	21	0.0328	15	8	0.0329	13	27	0.0066	15	34	0.0329
11	22	0.0197	15	15	0.0132	13	27.2	0.0066	15	35	0.1249
11	22.1	0.0066	15	16	0.0592	13	28.2	0.0329	15	35.3	0.0132
11	23	0.0328	15	17	0.0395	13	29	0.0197	15	36	0.0723
11	23.1	0.0066	15	18	0.0592	13	29.2	0.0263	15	37	0.0591
11	24	0.0525	15	19	0.0197	13	30	0.0197	15	38	0.0263
11	25	0.0066	15	20	0.0066	13	30.2	0.0394	15	38.3	0.0066
11	25.1	0.0066	16	8	0.0066	13	31	0.0329	15	39	0.0132
11	26	0.0328	16	15	0.0066	13	31.2	0.0329	15	39.3	0.0197
11	27	0.0263	16	16	0.0066	13	32	0.0329	15	40	0.0066
11	28	0.0132	16	17	0.0066	13	32.2	0.0394	15	40.3	0.0263
11	29	0.0197	17	16	0.0066	13	33	0.0066	15	41.3	0.0263
11	30	0.0132				13	33.2	0.0132	15	42.3	0.0132
11	31	0.0066				13	34.2	0.0066	16	32	0.0066
11	32	0.0066				14	28	0.0066	16	33	0.0132
11	33	0.0066				14	30	0.0066	16	34	0.0394
12	17.1	0.0132				14	30.2	0.0066	16	35	0.0197
12	18	0.0066				14	31	0.0066	16	36	0.046
12	19	0.0263				14	31.2	0.0263	16	37	0.0132
12	19.1	0.0066				14	32	0.0197	16	37.2	0.0066

Table 2 (*Continued*)

DXS8378	DXS10135	Haplotype frequencies	DXS7132	DXS10074	Haplotype frequencies	HPRTB	DXS10101	Haplotype frequencies	DXS7423	DXS10134	Haplotype frequencies
Allele	Allele		Allele	Allele		Allele	Allele		Allele	Allele	
12	21	0.0132				14	32.2	0.0197	16	38	0.0066
12	22	0.0066				14	33.2	0.0197	16	39	0.0066
12	23.1	0.0066				15	29.2	0.0066	16	39.3	0.0066
12	24	0.0066				15	30	0.0132	16	40	0.0066
12	25	0.0394				15	30.2	0.0066	16	41.3	0.0066
12	25.1	0.0066				15	32	0.0066	17	35	0.0066
12	26	0.0263				15	32.2	0.0066	17	36	0.0066
12	27	0.0132				15	34	0.0066	17	37.2	0.0066
12	28	0.0197				16	31	0.0066			
12	29	0.0132				16	32	0.0066			
12	31	0.0132									
12	34	0.0066									
13	20	0.0066									
13	21.1	0.0066									
13	22.1	0.0066									
13	24	0.0066									
13	26	0.0066									
14	23	0.0066									
15	24	0.0066									

antizyme 3 gene (*OAZ3*) [31] and the succinate dehydrogenase genes (*SDHA*) [32].

Aiming at modeling possible frequency-dependent balancing selection, phylogenetic relationships between haplotypes were reconstructed. Network topologies based on male haplotypes have shown only that distribution of certain haplotype variants is quite uniform among different populations. Only diagram constructed for DXS10074–DXS7132 pair depicts somewhat different outcome (Fig. 1). It reveals existence of two separate highly divergent clusters of haplotypes. Interestingly, one of the clusters represents haplotypes broadly distributed in Europe and Africa, yet Japanese population is completely absent there. Recombination seems unlikely explanation for the network topology since the distance within the Mentype Argus X-8 STR pairs is assumed to be <1 cM, whereas the pair to pair space is about 50 cM or more. Becker et al. [2] received high logarithm of the odds (LOD) scores for each pair (27–34) using 104 families consisting of three generations of grandfather, mother and grandsons. High mutation rates of X-STR loci seems to be another plausible explanation for the resulted network. However, mutation rates for the X-STRs included in the Mentype Argus X-8 kit do not appear substantially higher than those calculated for autosomal STRs, varying from 0.001199 (DXS10134) to 0.003564 (DXS7132) [2–4,33–42].

Table 3
Comparison of expected and observed homozygosity for 15 X-STR loci in Polish population based on Ewens–Watterson test. F_{exp} – expected homozygosity, F_{obs} – observed homozygosity, p – statisctical significance.

Locus	F_{exp}	F_{obs}	p
DXS9898	0.46804	0.22544	0.019
DXS6807	0.47344	0.30505	0.169
DXS101	0.25489	0.12294	0.007
DXS7133	0.39714	0.32114	0.360
DXS10011	0.08524	0.03989	0.000
DXS7424	0.38585	0.24158	0.129
DXS8377	0.19183	0.08176	0.000
DXS8378	0.42217	0.29873	0.242
HPRTB	0.39051	0.28001	0.244
DXS7423	0.5787	0.29837	0.028
DXS7132	0.46174	0.27414	0.112
DXS10134	0.16403	0.12318	0.245
DXS10074	0.29191	0.16426	0.072
DXS10101	0.19052	0.08064	0.000
DXS10135	0.12366	0.05953	0.000

One may rather suppose that present shape of haplotypes' networks results from thousand of years of constant evolutionary processes and preservation of such diversity may reflect the action of frequency dependent balancing selection. On the basis of Ewens–Watterson test, heterozygosity and phylogeny analyses one may expect frequency dependent selection to be the main force acting in increase of diversity of certain chromosome Xfragments. This led to accumulation of diversity in proximal neutral markers, such as X-STR loci analyzed in this study.

We therefore conclude that the unusual characteristics identified by neutrality tests and network analyses may reflect selective events in Europe and Africa, because the maintenance of two divergent haplotype clusters probably for a long time is most simply interpreted as resulting from the action of long-term balancing selection. However, present data can be also explained by demographic events that occurred during expansion of modern humans out of Africa. Under this neutral model, the second (African/European) X-cluster initially arose in Africa and later entered Southwest Asia, from where this cluster distributed throughout the rest of Europe. The absence of this cluster in East Asia can be explained by the effect of genetic drift. Alternatively, the origin of this cluster in Africa/Southwest Asia could have occurred much later, most probably during the expansion of first Neolithic farmers into Europe. Thus, additional data on X-chromosome haplotypic diversity in different populations of the world are required to clarify this question.

Hereby authors confirm that they have strictly followed the requirements stated in guideline [43] and the ISFG recommendations [44] in this article.

Table 4
Fst analysis results for populations from Poland (POL) [this study], Japan (JPN) [2], Ghana (GHA) [2], Hungary (HUN) [4] and Sweden (SWE) [3]. Data shown in the matrix below diagonal are Fst distances, and significance of p-values (<0.05) is given above diagonal. "+" denotes statistically significant result.

	POL	JPN	GHA	HUN	SWE
POL		+	+	+	+
JPN	0.00823		+	+	+
GHA	0.00644	0.00426		+	+
HUN	0.00108	0.00799	0.00623		+
SWE	0.00465	0.00304	0.00208	0.00573	

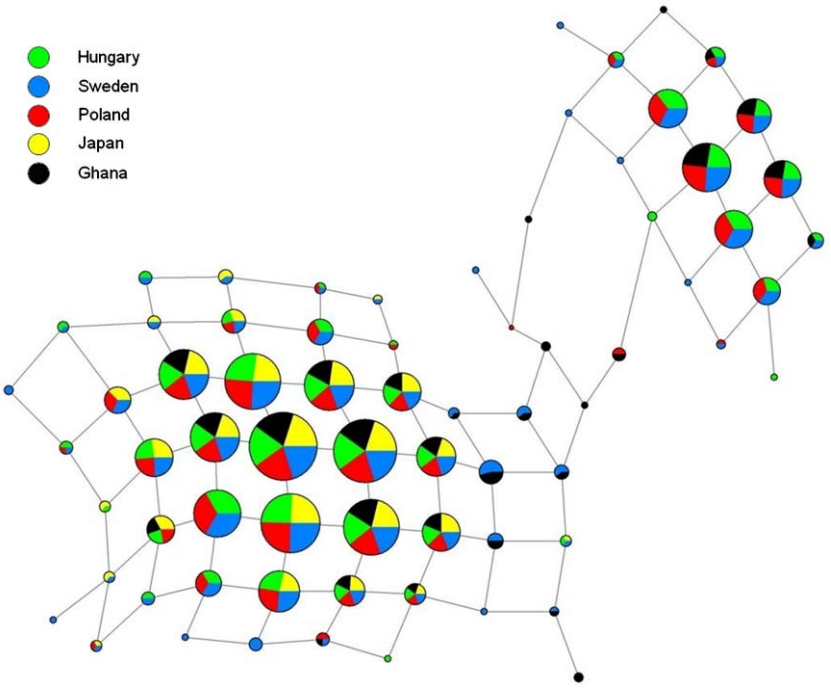

Fig. 1. Haplotypes' network for DXS10074–DXS7132 pair of loci.

Table 5
Comparison of pairwise linkage disequilibrium computation results based on haplotypes made up of loci included in the Mentype Argus X-8 kit. Statistical significance was set to 0.05. "+" denotes statistically significant result.

Locus	DXS8378	HPRTB	DXS7423	DXS7132	DXS10134	DXS10074	DXS10101	DXS10135
DXS8378		+	+	–	–	–	–	+
HPRTB	0.0445		–	–	–	–	–	–
DXS7423	0.0053	0.4056		–	–	–	–	–
DXS7132	0.6861	0.1896	0.3695		–	–	–	–
DXS10134	0.1848	0.3306	0.2992	0.3213		+	–	–
DXS10074	0.0966	0.1237	0.3280	0.1335	0.0000		–	–
DXS10101	0.7832	0.2834	0.4876	0.8439	0.9313	0.4953		–
DXS10135	0.0000	0.6449	0.7468	0.2527	0.6105	0.8530	0.5114	

References

[1] M. Poetsch, H. Petersmann, A. Repenning, E. Lignitz, Development of two pentaplex systems with X-chromosomal STR loci and their allele frequencies in a northeast German population, Forensic Sci. Int. 155 (2008) 71–76.

[2] D. Becker, H. Rodig, C. Augustin, J. Edelmann, F. Götz, S. Hering, R. Szibor, W. Brabetz, Population genetic evaluation of eight X-chromosomal short tandem repeat loci using Mentype Argus X-8 PCR amplification kit, Forensic Sci. Int. Genet. 2 (2008) 69–74.

[3] A.O. Tillmar, P. Mostad, T. Egeland, B. Lindblom, G. Holmlund, K. Montelius, Analysis of linkage and linkage disequilibrium for eight X-STR markers, Forensic Sci. Int. Genet. 3 (2008) 37–41.

[4] A. Zalán, A. Völgyi, W. Brabetz, D. Schleinitz, H. Pamjav, Hungarian population data of eight X-linked markers in four linkage groups, Forensic Sci. Int. 175 (2008) 73–78.

[5] V. Yotova, J.F. Lefebvre, O. Kohany, J. Jurka, R. Michalski, D. Modiano, G. Utermann, S.M. Williams, D. Labuda, Tracing genetic history of modern humans using X-chromosome lineages, Hum. Genet. 122 (2007) 431–443.

[6] R. Szibor, I. Plate, J. Edelmann, S. Hering, E. Kuhlisch, M. Michael, D. Krause, Chromosome X haplotyping in deficiency paternity testing principles and case report, Int. Congr. Ser. 1239 (2003) 815–820.

[7] C. Toni, S. Presciuttini, I. Spinetti, A. Rocchi, R. Domenici, Usefulness of X-chromosome markers in resolving relationships: report of a court case involving presumed half sisters, Int. Congr. Ser. 1288 (2006) 301–303.

[8] R. Ploski, M. Wozniak, R. Pawlowski, D.M. Monies, W. Branicki, T. Kupiec, A. Kloosterman, T. Dobosz, E. Bosch, M. Nowak, R. Lessig, M.A. Jobling, L. Roewer, M. Kayser, Homogeneity and distinctiveness of Polish paternal lineages revealed by Y chromosome microsatellite haplotype analysis, Hum. Genet. 110 (2002) 592–600.

[9] M. Wozniak, T. Grzybowski, J. Starzynski, T. Marciniak, Continuity of Y chromosome haplotypes in the population of Southern Poland before and after the Second World War, Forensic. Sci. Int. Genet. 1 (2007) 134–140.

[10] M. Nelis, T. Esko, R. Mägi, F. Zimprich, A. Zimprich, D. Toncheva, S. Karachanak, T. Piskácková, I. Balascák, L. Peltonen, E. Jakkula, K. Rehnström, M. Lathrop, S. Heath, P. Galan, S. Schreiber, T. Meitinger, A. Pfeufer, H.E. Wichmann, B. Melegh, N. Polgár, D. Toniolo, P. Gasparini, P. D'Adamo, J. Klovins, L. Nikitina-Zake, V. Kucinskas, J. Kasnauskiene, J. Lubinski, T. Debniak, S. Limborska, A. Khrunin, X. Estivill, R. Rabionet, S. Marsal, A. Julià, S.E. Antonarakis, S. Deutsch, C. Borel, H. Attar, M. Gagnebin, M. Macek, M. Krawczak, M. Remm, A. Metspalu, Genetic structure of Europeans: a view from the North-East, PLoS ONE 4 (2009) e5472, doi:10.1371/journal.pone.0005472.

[11] R. Szibor, J. Edelmann, S. Hering, I. Plate, H. Wittig, L. Roewer, P. Wiegand, F. Calì, V. Romano, M. Michael, Cell line DNA typing in forensic genetics – the necessity of reliable standards, Forensic Sci. Int. 138 (2003) 37–43.

[12] www.chrx-str.org.

[13] S.D.E. Park, Trypanotolerance in West African cattle and the population genetic effects of selection, PhD thesis, University of Dublin, 2001.

[14] L. Excoffier, G. Laval, S. Schneider, Arlequin ver 3. 0: an integrated software package for population genetics data analysis, Evol. Bioinform. Online 1 (2005) 47–50.

[15] Y. Hochberg, A sharper Bonferroni procedure for multiple test of significance, Biometrika 75 (1988) 800–802.

[16] J. Krüger, W. Fuhrmann, K. Lichte, C. Steffens, On the utilization of erythrocyte acid phosphatase polymorphism in paternity evaluation, Dtsch. Z. Gesamte. Gerichtl. Med. 64 (1968) 127–146.

[17] T. Kishida, W. Wang, M. Fukuda, Y. Tamaki, Duplex PCR of the Y-27H39 and HPRT loci with reference to Japanese population data on the HPRT locus, Nihon Hoigaku Zasshi 51 (1997) 67–69.

[18] D. Desmarais, Y. Zhong, R. Chakraborty, C. Perreault, L. Busque, Development of a highly polymorphic STR marker for identity testing purposes at the human androgen receptor gene (HUMARA), J. Forensic Sci. 43 (1998) 1046–1049.

[19] H.-J. Bandelt, P. Forster, A. Röhl, Median-joining networks for inferring intraspecific phylogenies, Mol. Biol. Evol. 16 (1999) 37–48.

[20] H.B. Luo, Y. Ye, Y.Y. Wang, W.B. Liang, L.B. Yun, M. Liao, J. Yan, J. Wu, Y.B. Li, Y.P. Hou, Characteristics of eight X-STR loci for forensic purposes in the Chinese population, Int. J. Legal Med. (2009), doi:10.1007/s00414-009-0386-z.
[21] W. Branicki, P. Wolańska-Nowak, A. Parys-Proszek, T. Kupiec, Application of the Mentype Argus x-8 kit to forensic casework, Probl. Forensic Sci. LXXIII (2008) 53–64.
[22] C. Bini, S. Ceccardi, G. Ferri, S. Pelotti, M. Alu, E. Roncaglia, G. Beduschi, L. Caenazzo, E. Ponzano, P. Tasinato, C. Turcji, L. Buscemi, M. Mazzanti, A. Tagliabracci, C. Toni, I. Spinetti, R. Domenici, S. Presciuttini, Development of a heptaplex PCR system to analyse X-chromosome STR loci from five Italian population samples. A collaborative study, Forensic Sci. Int. 153 (2005) 231–236.
[23] M. Aler, P. Sánchez-Diz, I. Gomes, M. Gisbert, A. Carracedo, A. Amorim, L. Gusmão, Genetic data of 10 X-STRs in a Spanish population sample, Forensic Sci. Int. 173 (2007) 193–196.
[24] K.A. Tabbada, M.C. De Ungria, L.P. Faustino, D. Athanasiadou, B. Stradmann-Bellinghausen, P.M. Schneider, Development of a pentaplex X-chromosomal short tandem repeat typing system and population genetic studies, Forensic Sci. Int. 154 (2005) 173–180.
[25] S. Hering, N. Brundirs, E. Kuhlisch, J. Edelmann, I. Plate, M. Benecke, P.H. Van, M. Michael, R. Szibor, DXS10011: studies on structure, allele distribution in three populations and genetic linkage to further q-telomeric chromosome X markers, Int. J. Legal Med. 118 (2004) 313–319.
[26] A. Tamura, M. Iwata, I. Takase, T. Miyazaki, K. Matsui, H. Nishio, K. Suzuki, Analysis of two types of novel alleles in the DXS10011 locus, Leg. Med. (Tokyo) 6 (2004) 52–54.
[27] E.J. Hollox, J. Armour, Directional and balancing selection in human beta-defensins, BMC Evol. Biol. 8 (2008) 113, doi:10.1186/1471-2148-8-113.
[28] F. He, D.-D. Wu, Q.-P. Kong, Y.-P. Zhang, Intriguing balancing selection on the intron 5 region of LMBR1 in human population, PLoS ONE 3 (2008) e2948, doi:10.1371/journal.pone.0002948.
[29] B. Tu, S.J. Mack, A. Lazaro, A. Lancaster, G. Thomson, K. Cao, M. Chen, R. Ling, R. Hartzman, J. Ng, C.K. Hurley, HLA-A,-B,-C,-DRB1 allele and haplotype frequencies in an African American population, Tissue Antigens 69 (2006) 73–85.
[30] F. Calafell, F. Roubinet, A. Ramírez-Soriano, N. Saitou, J. Bertranpetit, A. Blancher, Evolutionary dynamics of the human ABO gene, Hum. Genet. 124 (2008) 123–135.
[31] G.L. Christensen, I.P. Ivanov, S.P. Wooding, J.F. Atkins, A. Mielnik, P.N. Schlegel, D.T. Carrell, Identification of polymorphisms and balancing selection in the male infertility candidate gene, ornithine decarboxylase antizyme 3, BMC Med. Genet. 7 (27) (2006), doi:10.1186/1471-2350-7-27.
[32] B.E. Baysal, E.C. Lawrence, R.E. Ferrell, Sequence variation in human succinate dehydrogenase genes: evidence for long-term balancing selection on SDHA, BMC Biol. 5 (12) (2007), doi:10.1186/1741-7007-5-12.
[33] J. Janica, W. Pepiński, M. Skawrońska, A. Niemcunowicz-Janica, E. Koc-Zurawska, I. Sołtyszewski, Polymorphism of four X-chromosomal STRs in a population sample of Belarusian minority residing in Podlasie (NE poland), Arch. Med. Sadowej Kryminol. 56 (2006) 232–235.
[34] Q.L. Liu, D.J. Lv, X.L. Wu, H.Y. Sun, X.Y. Wu, H.L. Lu, Development of a five ChX STRs loci typing system, Int. J. Legal Med. 122 (2008) 261–265.
[35] W. Pepiński, M. Skawrońska, A. Niemcunowicz-Janica, E. Koc-Zórawska, J. Janica, Polymorphism of four X-chromosomal STRs in a population sample of Podlasie (NE Poland), Arch. Med. Sadowej Kryminol. 55 (2005) 154–156.
[36] W. Pepinski, A. Niemcunowicz-Janica, M. Skawronska, E. Koc-Zorawska, J. Janica, J. Berent, I. Soltyszewski, X-chromosomal polymorphism data for the ethnic minority of Polish Tatars and the religious minority of old believers residing in northeastern Poland, Forensic Sci. Int. Genet. 1 (2007) 212–214.
[37] A. Pico, A. Castillo, C. Vargas, A. Amorim, L. Gusmão, Genetic profile characterization and segregation analysis of 10 X-STRs in a sample from Santander, Colombia, Int. J. Legal Med. 122 (2008) 347–351.
[38] J.Y. Son, Y.S. Lee, C.M. Choung, S.D. Lee, Polymorphism of nine X chromosomal STR loci in Koreans, Int. J. Legal Med. 116 (2002) 317–321.
[39] M.A. Tariq, O. Ullah, S.A. Riazuddin, S. Riazuddin, Allele frequency distribution of 13 X-chromosomal STR loci in Pakistani population, Int. J. Legal Med. 122 (2008) 525–528.
[40] S. Turrina, R. Atzei, G. Filippini, D. De Leo, Development and forensic validation of a new multiplex PCR assay with 12 X-chromosomal short tandem repeats, Forensic Sci. Int. Genet. 1 (2007) 201–204.
[41] A. Carracedo, J.M. Butler, L. Gusmao, W. Parson, L. Roewer, P.M. Schneider, Publication of population data for forensic purposes, Forensic Sci. Int. Genet. 4 (2010) 145–147.
[42] P.M. Schneider, Scientific standards for studies in forensic genetics, Forensic Sci. Int. 165 (2007) 238–243.
[43] J. Edelmann, S. Hering, C. Augustin, R. Szibor, Characterisation of the STR markers DXS10146, DXS10134 and DXS10147 located within a 79.1 kb region at Xq28, Forensic Sci. Int. Genet. 2 (2008) 41–46.
[44] H. Asamura, H. Sakai, M. Ota, H. Fukushima, Japanese population data for eight X-STR loci using two new quadruplex systems, Int. J. Legal Med. 120 (2006) 303–309.